KARNATAKA STATE AKKAMAHADEVI WOMEN'S UNIVERSITY, VIJAYAPURA

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Evaluation of Nutraceutical and Biological Properties Of *Cayratia mollissima* - An Endangered Species Of Western Ghats Of India

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Chapter I
Introduction

Traditional Food

Traditional Indian foods have been prepared for many years and preparation varies across the country. Traditional wisdom about processing of food, its preservation techniques, and their therapeutic effects have been established for many generations in Indian Food systems can deliver numerous biological functions through dietary components in the human body. Indian traditional foods are also recognized as functional foods because of the presence of functional components such as body-healing chemicals, antioxidants, dietary fibres, and probiotics. These functional molecules help in weight management, and blood sugar level balance and support immunity of the body. The functional properties of foods are further enhanced by processing techniques such as sprouting, malting, and fermentation (Hotz et al. 2007).

Traditionally, Indian foods are classified into three main categories. Cooked vegetables, milk, fresh fruits, and honey are meant for the truly wise and are considered as Satvika foods. Foods that bring out the lowest, crass qualities of human behaviour such as meat, liquor, garlic, and spicy and sour foods are classified as Tamasika foods. Foods that give enough energy to carry out daily work are categorized as Rajsika foods (Dubey, 2010). Aryans classified food materials on the basis of their nature and use such as Sukhadhanya cereals), Samidhanya (pulses), Phala (fruits), Shakna (vegatables), Payovarga (milk products), Madhyavarga (alcoholic beverages) and Mamasavarga (animal products) (Achaya, 1994)

Phytochemicals in Medicinal plants and Human health

Medicinal plants have played an essential role in the development of human culture, for example religions and different ceremonies. Many of the modern medicines are produced indirectly from medicinal plants, for example aspirin. Many food crops have medicinal effects,

for example garlic. Medicinal plants are resources of new drugs. It is estimated there are more than 250, 000 flower plant species. Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons. Cultivation and preservation of medicinal plants protect biological diversity, for example metabolic engineering of plants. The medicinal effects of plants are due to metabolites especially secondary compounds produced by plant species. Plant metabolites include: primary metabolites and secondary metabolites Phytotherapy is the use of plants or plant extracts for medicinal purposes (especially plants that are not part of the normal diet). Phytochemistry is the study of phytochemicals produced in plants, describing the isolation, purification, identification, and structure of the large number of secondary metabolic compounds found in plants.

Endangered plant species in India

India has a rich resource base of medicinal plants, plush with about 8,000 different species. According to the Government of India (GoI), traditional medicines are the sole means of health care for about 65 percent of the population. The medicinal plants are basic raw material for the production of Ayurveda and Unani medicines. The bulk of the raw material (about 80% of the demand) is derived from the forests only. Hence, the forest areas have been over exploited in the past to meet the requirement of the pharmaceutical and allied industries. Consequently, many of the important plant species have been threatened and some of them are on the verge of extension due to unscientific collection by untrained persons. In recent years, medicinal plants have also been gaining immense popularity not only in developing countries but also in developed countries due to various well-known reasons like side effects of synthetic drugs.

According World Health Organization (WHO), traditional medicine refers to the sum total of knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve, or

treat physical and mental illnesses (WHO, 2008). Traditional medical practices can include plant, animal, and mineral-based medicines, massage, spiritual therapies, and a variety of other techniques unique to different regions and cultures (WHO, 2005). Traditional medicine is typically contrasted with conventional medicine, also referred to as allopathic, modern, orthodox, or Western medicine, which is based on biochemical theories of illness (WHO 2008, WHO 2005).

Chapter II
Review of Literature

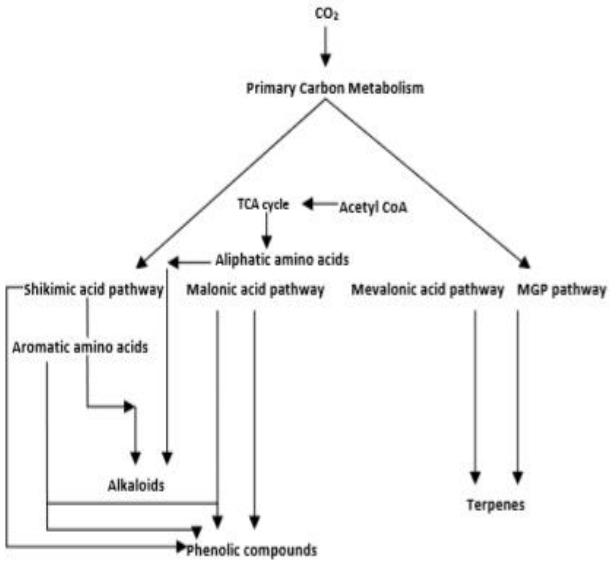
Natural products are a source of new chemical diversity and are the choice of today's world. The sources of natural product are plants, animals and microorganisms. Among them plant and its products are more reliable for its renewability and therefore, considered as catalyst for human welfare. Still, they are the primarily required materials for health care system in some parts of the world. Therefore, in the past few decades, there is a growing research interest in plants as a therapeutic agent. The therapeutic potential of plant products can be traced back to over five thousand years ago as there is evidence of its use in the treatment of diseases and for revitalizing body systems in Indian, Egyptian, Chinese, Greek and Roman civilizations (Mahesh and Satish, 2008). Also India is one of the mega diversity hot spots with rich heritage of traditional knowledge of folk medicines. Therefore in India, plants of therapeutic potential are widely used by all sections of people both as folk medicines in different indigenous systems of medicine like Siddha, Ayurveda and Unani and also as processed product of pharmaceutical industry (Srinivasan et al. 2007). India has about 4.5 million plant species and among them estimated only 250,000- 500,000 plant species, have been investigated phytochemically for biological or pharmacological activity. Still a large number of higher plants as a source for new therapeutics are to be explored. The potential for developing phytomedicine into various health care products appears rewarding, both from the perspective of economy and safety. Friedman et al. (2007) and Serafinao et al. (2008) noted that many plant extracts are quite effective than the synthetic ones with no or insignificant side effects and very little scientific research on their biological activity has been worked out. Furthermore, the growth of multidrug resistant microbial strains which are limiting the effectiveness of synthetic drugs led to an awareness among the common people against the use of synthetic product (Sundsford 2004; Edith et al. 2005; Hancock 2005; Isturiz 2008). This factor forced the scientists to develop methods of

bioprocesses for the production and extraction of compounds from natural renewable sources for their potential application in food, cosmetics and pharmaceuticals industry with antimicrobial, antioxidant, anti-inflammatory and anti-diabetic activity (Velioglu et al. 1998; Oreopoulou and Tzia 2007). Nayak and Pereira (2006) proposed that the therapeutic properties of plant extracts have far more to offer in future as a novel discovery Thus, use of phytochemicals as major bioactive compounds with multidimensional benefits are gaining momentum.

Phenolics the important secondary metabolites from plants:

Plant metabolites represent an important source of sugars, minerals, organic acids, dietary fibre, as well as bioactive compounds like phenols, alkaloids and flavanoids which are synthesized as secondary metabolites and are constitutive, existing in healthy plants in their biologically active forms, but others occur as inactive precursors and are activated in response to tissue damage or pathogen attack (Osbourne 1996; Dahanukar et al. 2000). These secondary metabolites produced by plants are with wide range of chemical, physical and biological activities. The majority of bioactive compounds belong to a number of families, each of which has particular structural characteristics. There are four major pathways for synthesis of secondary metabolites or bioactive compounds: (1) Shikimic acid pathway, (2) Malonic acid pathway, (3) Mevalonic acid pathway and (4) Non-mevalonate (MEP) pathway (Tiaz and Zeiger, 2006). Alkaloids are produced by aromatic amino acids (shikimic acid pathway) and by aliphatic amino acids (tricarboxylic acid cycle). Phenolic compounds are synthesized through shikimic acid pathway and malonic acid pathway. Through mevalonic acid pathway and MEP pathway terpenes are produced. Simplified illustrations of different pathways for the production of three major groups of plant bioactive compounds are shown in Fig 1.

Fig 1: A simplified view of pathways for production of three major groups of bioactive compounds of plant resource (Tiaz and Zeiger, 2006)



Of these, the bioactive compounds mostly found in plants are phenolics and polyphenols (Shi et al. 2005) which are secondary metabolites and a much diversified group. Important subclasses in this group of compounds which have been found to have antimicrobial activity include phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins. This group includes metabolites derived from the condensation of acetate units (tepernoids) those produced by the modification of aromatic amino acids (phenylpropanoids and coumarins), flavonoids, isoflavonoids and tannins. Phenolics are involved in many physiological activities such as stimulation of phagocytic cells, host-mediated tumor activity. They are soluble in water, alcohol and acetone and gives precipitates with proteins (Basri and Fan 2005). There are evidences that phenolics were traditionally used for protection of inflamed surfaces of the mouth and treatment of wounds, hemorrhoids and diarrhoea (Ogunleye and Ibitoye 2003).

Cayratia mollissima

Exploitation of new and diverse sources of variation is needed to combat various biotic and abiotic stresses. In the collection of wild grape germplasm, different species of *Cayratia* (Family Vitaceae) were studied after collecting them from various sources of Indian forests. Some of the species of *Cayratia* are rare and endemic to N.E. India and Andaman. These species are resistant to major fungal diseases as well as tolerant to insect pests. Phyto-chemicals recorded which have importance in preparations of Ayurvedic medicines, homemade remedies, natural pesticides and classification of plants. Information generated through study has significant importance in conservation of plant biodiversity and utilization of wild resources of cultivated grapes in grape improvement programme with the help of advanced tools of biotechnology.

The natural habitats of *Cayratia mollissima* species include the fringes and under torey of lowland dipterocarp forest, the fringes of hill dipterocarp forest, and limestone vegetation (Latiff, 1981). Its geographical range includes India, Myanmar, Thailand, Vietnam, Kampuchea, and the Malay Peninsula (Latiff, 1981).

Chapter II
Objectives

The Objectives carried out through this research are:

1. Collection and identification of the *Cayratia mollissima* fruit from the western Ghats of South India
2. Examining the physical properties of fresh and dried fruit sample of *Cayratia mollissima*
3. Proximate analysis of the fruit sample of *Cayratia mollissima*
4. Identification of phytoconstitutents of *Cayratia mollissima* (various extracts) by Qualitative analysis
5. *In vitro* Antimicrobial activity study of different extracts of *Cayratia mollissima* fruit sample by disc diffusion method
6. Determination of the antioxidant activity of the different extracts of the *Cayratia mollissima* fruit
7. *In vitro* study of anticancer activity of *Cayratia mollissima* fruit sample (Various extracts) against various cancer Cell Lines.

Chapter IV
Methodology

The present Study follows the below mentioned Study design, methodology and Analysis techniques:

1. **Collection and identification of fruit sample:** Western Ghats of India is regarded as one of hot spots in the world having rich plant diversity. Medicinal plants which find in this unique niche, diverse habitat, bearing antimicrobial and ethno botanical properties will be selected. Currently, *Cayratia mollissima* fruit will be identified in the field survey and selected for the research study for exploring its nutritional values.
2. **Physical Analysis:**
    - Total weight of the fresh and dried fruit
    - Pulp weight of the fresh and dried fruit
    - Length of the fresh and dried fruit
    - Width of the fresh and dried fruit
    - Number of seeds present in the fresh and dried fruit
    - Seed weight in the fresh and dried fruit
3. **Proximate analysis of the fruit sample of *Cayratia mollissima***

The nutritional composition of the fruit sample will be analysed by following the standard food analysis methods described in the Association of Official Analytical Chemists (AOAC, 2000).

- Estimation of moisture
- Estimation of Ash
- Estimation of Crude fiber
- Estimation of total lipids

- Estimation of Protein
- Estimation of CHO

Estimation of minerals

- Estimation of minerals.

a. The total phenolic content of the different plant extracts will be determined according to Folin-Ciocalteu procedure (Singleton and Rossi, 1965). The total phenolic content will be calculated as gallic acid equivalent (GAE) in mg/g dry weight of extract (DE).

b. Estimation of water soluble vitamins

4. Phytochemical screening

After extraction phytochemical screening assay can be done which helps us in preliminary detection of specific compound(s) present in the extract mixture or fraction (Table 2).

Table 2: Phytochemical screening methods

Secondary metabolite	Test performed	Reference
Alkaloid	Dragendorff's test	Kumar *et al.* 2007
Anthraquinone	Borntrager's test	Kumar *et al.* 2007
Cardiac glycosides	Kellar – Kiliani test	Parekh and Chanda 2007.
Flavonoid	Shinoda test	Kumar *et al.* 2007.
Phenol	Phenol test	Kumar *et al.* 2007.
Phlobatannin	-	Edeoga *et al.* 2005.
Pyrrolizidine alkaloid	-	McGaw *et al.* 2007
Reducing sugar	Fehling test	Akinyemi *et al.* 2005
Saponin	Frothing test /Foam test	Parekh and Chanda 2007.
Steroid	Liebermann-Burchardt test	Kumar *et al.* 2007.
Tannin	Braemer's test	Parekh and Chanda 2007.
Terpenoid	Salkowski test	Edeoga *et al.* 2005.

5. *In vitro* Antimicrobial activity study of different extracts of *Cayratia mollissima* fruit sample by disc diffusion method

Disc diffusion assay: Antimicrobial activity of the fruit extract will be carried out according to the protocol of Clinical Laboratory Standards Institute (CLSI, 2011) documents M02-A11 (for test bacteria), M51-P (for test fungi).

6. Determination of the antioxidant activity of the different extracts of the *Cayratia mollissima* fruit

Antioxidant Activity: One gram of plant material will be taken and 20 ml each solvent (cooked, methanol and 70% aq. ethanol) and extraction was achieved with agitation for 18 to 24 hr at ambient temperature. The fruit extracts were filtered, diluted up to 25 ml and antioxidant properties will be analysed.

a. Ferric reducing antioxidant power (FRAP) assay will be carried out using the method described by Benzie and Strain (1996). The ability of the fruit extract to reduce ferric ions of sample was the base of its antioxidant capacity and it was calculated as Trolox equivalent (TE) in mg/g dry extract.

b. F**ree radical scavenging activity** of the fruit samples will be determined using the stable radical DPPH (1,1-diphenyl-2-picrylhydrazyl) (Blois, 1958). The 2,20-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation (ABTS.þ)-scavenging activity will be measured according to the method described by Re et al. (1999).

c. **Phosphomolybdenum Assay** The antioxidant activity of fruit extracts will be evaluated by phosphomolybdenum method according to the procedure mentioned by Prieto et al. (2005).

d. **H_2O_2 Scavenging Assay**: Hydrogen peroxide (H_2O_2) scavenging activity of plant extracts is evaluated by measuring the disappearance of H_2O_2 at a wavelength of 230 nm as described by Ruch et al. (1989).

7. **Anticancer Activity Studies**: *In vitro* anticancer activity of the selected extracts will be performed by assessing cytotoxicity studies in selected cancer cell lines.

Chapter V:
Significance of the study

Cayratia mollissima growing in the natural habitats are being used as the food sources to fulfil the daily requirements of important nutrients by the rural people and this fruit is considered as one of the cheapest sources of energy for human consumption. This fruit is essentially important in the biochemical and nutritional aspects might be the rich sources of minerals, proteins, amino acids, carbohydrates, fibres, vitamins and other bioactive compounds especially the antioxidant compounds which on consumption contribute several health benefits against various diseases.

Chapter VI:
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